

Difference in severity of porcine circovirus type two-induced pathological lesions between Landrace and Pietrain pigs¹

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ABSTRACT: Anecdotal information from the field suggests that there are host genetic differences in susceptibility to porcine circovirus type 2 (PCV2) associated disease among Landrace and Pietrain breeds. The objective of this study was to determine if a difference exists in PCV2 susceptibility between Landrace and Pietrain pigs under experimental conditions. Thirty-nine Landrace pigs and 39 Pietrain pigs were blocked by breed, sire, dam, and litter and randomly divided into the following 4 groups: Landrace noninoculated negative control (Landrace-NEG; $n = 13$), Pietrain noninoculated negative control (Pietrain-NEG; $n = 13$), Landrace-PCV2 ($n = 26$; Landrace), and Pietrain-PCV2 ($n = 26$; Pietrain). After waning of passively acquired anti-PCV2 antibodies, Landrace-PCV2 and Pietrain-PCV2 groups were inoculated with PCV2 isolate ISU-40895. The Landrace-NEG and Pietrain-NEG groups were housed in a separate room, remained noninoculated, and served as negative controls. All pigs in all groups

were necropsied at 21 d post PCV2-inoculation. Onset of seroconversion and concentrations of anti-PCV2-IgM, anti-PCV2-IgG, and anti-PCV2 neutralizing antibodies were similar in Landrace-PCV2 and Pietrain-PCV2 groups. Furthermore, the amount of PCV2 DNA and cytokine concentrations in serum and plasma samples were not different between the 2 PCV2-inoculated groups. The severity of PCV2-associated microscopic lesions was different between Landrace and Pietrain pigs; Landrace-PCV2 pigs had significantly ($P < 0.05$) more severe lymphoid lesions than the Pietrain-PCV2 pigs. Although the pigs originated from the same farm where their dams were commingled, passively acquired anti-PCV2-antibodies waned in Pietrain pigs by approximately 12 wk of age, whereas the majority of the Landrace pigs remained PCV2 seropositive until 18 wk of age and beyond. The results from this study indicate that a genetic difference exists between these 2 breeds of pigs in susceptibility to PCV2-associated lesions.

Key words: breed, conventional pig model, genetic susceptibility, Landrace, Pietrain, porcine circovirus type 2

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INTRODUCTION

Porcine circovirus type 2 (PCV2) infection is characterized by distinct lymphoid lesions such as depletion of lymphocytes and granulomatous inflammation (Sorden, 2000). Large amounts of PCV2 antigen or nucleic acids can be demonstrated in the cytoplasm of macrophages and dendritic cells replacing lymphocytes in the depleted follicles (Allan and Ellis, 2000). Disease

syndromes associated with PCV2 infection, including systemic infection or postweaning multisystemic wasting syndrome (PMWS), respiratory disease, reproductive disorders, and enteric disease are summarized as PCV2-associated disease (PCVAD; Opriessnig et al., 2007).

Anecdotal reports from the field suggest Landrace pigs are more susceptible to PCV2-infection and PCVAD compared with Pietrain pigs. However, controlled genetic studies on susceptibility to specific diseases are difficult to conduct due to size and cost limitations resulting in restrictions on pigs per line and breed. It is well known that genetic components in disease resistance exist in pigs such as reported with porcine reproductive and respiratory syndrome virus (Halbur et al., 1998; Petry et al., 2005, 2007; Vincent et al., 2006),

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pseudorabies virus (Reiner et al., 2002), *Salmonella* sp. (Wigley, 2004), and *Escherichia coli* (Duchet-Suchaux et al., 1991; Michaels et al., 1994).

Spanish scientists observed a trend toward genetic susceptibility and PCVAD in field studies involving certain paternal genetic backgrounds (López-Soria et al., 2004; Sibila et al., 2005). In a controlled trial, Landrace pigs were found to develop more severe microscopic PCV2-associated lesions compared with Large White and Duroc pigs (Opriessnig et al., 2006a). However, a French group was not able to confirm the improvement of PCVAD using selected breeding with Pietrain sires (Rose et al., 2004).

The objective of the current study was to determine if a difference in severity of PCV2-induced pathological lesions exists between conventional Landrace and Pietrain pigs.

MATERIALS AND METHODS

The experimental protocol in this study was approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals

A source herd for purebred Landrace and Pietrain pigs was identified. Based on routine serological testing, the source herd was negative for porcine reproductive and respiratory syndrome virus and swine influenza virus but positive for PCV2. However, PCVAD was not observed in the source herd or on the farms supplied with offspring from this herd. To fulfill study requirements, the following selective breeding was implemented: A total of 13 Landrace sows and 13 Pietrain sows were used. For each breed, 6 different nonrelated sires were utilized resulting in 2 to 3 dams per sire and breed. From each litter, 3 random piglets were selected for the study resulting in a total of 39 Landrace piglets and 39 Pietrain piglets. Of the Landrace piglets, 20 were male and 19 were female; of the Pietrain piglets, 19 were male and 20 were female. The piglets of both breeds were born in the same barn under the same environmental and management conditions, were allowed to suckle colostrum, and were weaned at 3 wk of age, at which time they were brought to the research facility at Iowa State University.

Experimental Design and Housing

All pigs were commingled and raised in the same building and room at the research facility and were periodically monitored for presence of PCV2-antibodies. After waning of the maternal antibodies at approximately 21 wk of age, the pigs were blocked by litter and were distributed to 1 of 3 identical rooms. One pig from each litter served as a noninoculated negative control (**Landrace-NEG**, $n = 13$; **Pietrain-NEG**, n

$= 13$), whereas the remaining 2 pigs from each litter were inoculated with PCV2 (**Landrace-PCV2**, $n = 26$; **Pietrain-PCV2**, $n = 26$). Pietrain and Landrace pigs were randomly distributed across pens in each room and commingled within pens. The pens were 2.5×3.6 m raised wire decks, which were equipped with 1 nipple drinker and 1 self-feeder. All groups were fed ad libitum a balanced, pelleted, complete feed ration free of animal proteins and antibiotics (Nature's Made, Heartland Coop, Prairie City, IA).

The Landrace-PCV2 and Pietrain-PCV2 groups were inoculated with PCV2 at 21 wk of age. The Landrace-NEG and Pietrain-NEG groups remained noninoculated. After PCV2-inoculation, pigs were bled weekly for 3 wk. Serum samples were tested for the presence of anti-PCV2-IgG and -IgM antibodies, neutralizing antibodies and PCV2 associated viremia, and plasma samples were tested for presence of selected cytokines. All pigs in all groups were necropsied at 21 d postinoculation (**DPI**).

Inoculation

The PCV2-isolate ISU-40895 (GenBank accession number AF264042) originally isolated in 1998 from a 40-kg pig suffering from PMWS on an Iowa farm (Fenaux et al., 2000) was used for the inoculation. At 21 wk of age, all Landrace-PCV2 and Pietrain-PCV2 pigs each received 5 mL ($10^{4.5}$ TCID₅₀/mL) of ISU-40895 PCV2 inoculum: 3 mL intranasally and 2 mL intramuscularly.

Anti-PCV2-IgG and -IgM Antibodies

Blood [12.5-mL Corvac serum separator tube (Tyco Healthcare Group LP, Mansfield, MA) and 7-mL BD Vacutainer tube containing 12 mg of K₃ EDTA (BD, Franklin Lakes, NJ)] was collected periodically during the growing phase, on the day of inoculation, and on 7, 14, and 21 DPI. Serum samples were tested by an ORF2-based PCV2 IgG ELISA as described previously (Nawagitgul et al., 2002) and by the IgM Ingezim PCV ELISA (Ingenasa, Madrid, Spain). For the IgG ELISA, serum samples were considered positive if the calculated sample-to-positive (**S/P**) ratio was 0.2 or greater (Nawagitgul et al., 2002). For the IgM-ELISA, serum samples were considered positive on the IgM Ingezim PCV ELISA if the optical density (**OD**) value was greater than the OD 450-nm value of the positive control $\times 0.4$ as recommended by the manufacturer.

Neutralizing PCV2 Antibodies

A fluorescence focus neutralization assay was done on serum samples collected on the day of inoculation and at 7, 14, and 21 DPI to determine the presence of neutralizing antibodies against PCV2 according to the standard Iowa State University Veterinary Diag-

nostic Laboratory operating protocol (Pogranichnyy et al., 2000). All samples were retested 3 times to confirm repeatability. The assay was performed with PCV2 isolate ISU-98-15237.

PCV2 DNA Quantification

The DNA extraction on serum samples collected on the day of inoculation and at 7, 14, and 21 DPI was performed using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). The DNA extracts were used for quantification of the PCV2 genomic DNA copy numbers by real-time PCR (Opriessnig et al., 2003).

Cytokine Assays

Plasma samples collected at the day of inoculation and at 7, 14, and 21 DPI were tested by ELISA for presence of interferon (IFN)- γ , IL-10 (Invitrogen/Biosource, Carlsbad, CA), and IFN- α (PBL Biomedical Laboratories, Piscataway, NJ; Petry et al., 2007). All assays were performed according to the specifications of the manufacturers. Samples were run at 1:10, 1:4, or 1:2 dilutions (or a combination of these) of plasma (assay dependent), respectively.

Clinical Evaluation and Necropsy

After PCV2 inoculation, the pigs were evaluated daily for clinical signs including respiratory disease, enteric disease, and behavioral changes such as lethargy and inappetence. All pigs were necropsied at 24 wk of age (21 d post PCV2 inoculation). Macroscopic lung lesions, scored from 0 to 100% of the lung affected, and the size of lymph nodes, scored from 0 (normal) to 3 (4 times the normal size), were estimated in a blinded fashion as described previously (Opriessnig et al., 2006a). Lungs were insufflated with fixative. Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric), tonsil, thymus, ileum, kidney, colon, spleen, and liver were collected at necropsy and fixed in 10% neutral-buffered formalin and routinely processed for histological examination.

Histopathology

Microscopic lesions were evaluated in a blinded fashion. Lung sections were scored for the presence and severity of interstitial pneumonia ranging from 0 (normal) to 6 (severe diffuse) as described previously (Halbur et al., 1995). Sections of heart, liver, kidney, brain, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 (none) to 3 (severe). Lymphoid tissues including lymph nodes, tonsil, and spleen were evaluated for the presence of lymphoid depletion ranging from 0 (normal) to 3 (severe) and histiocytic inflammation and replacement of follicles ranging from 0 (normal) to 3 (severe; Opriessnig et al., 2004a).

Immunohistochemistry

The immunohistochemistry (IHC) for detection of PCV2-specific antigen was performed on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, spleen, Peyer's patches, and thymus using a rabbit polyclonal antiserum as described previously (Sorden et al., 1999). The PCV2-antigen scoring was done in a blinded fashion, and scores ranged from 0 = no signal to 3 = more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining (Opriessnig et al., 2004a).

Overall Microscopic Lymphoid Lesion Score

The overall microscopic lymphoid lesions score, which accounts for lymphoid depletion, histiocytic inflammation, and PCV2-antigen present in lymphoid tissues were calculated for each pig as described previously (Opriessnig et al., 2004a) and ranged from 0 = normal to 9 = severe. Pigs were grouped into 4 categories on the basis of the overall microscopic lymphoid lesion score: normal (score = 0), mild (score = 1 to 3), moderate (score = 4 to 6), and severe (score = 7 to 9).

Statistical Analysis

Summary statistics were calculated for all groups to assess the overall quality of the data, including normality. Repeated measures multivariate ANOVA was performed using the general linear model (GLM) function (SAS Institute Inc., Cary, NC) for assessment of continuous data with the null hypothesis of no differences over time between group means. For the model, the dependent factor was \log_{10} genomic copies/mL, anti-IgG sample-to-positive ratio, anti-IgM OD value, or the fluorescence focus neutralization assay titer. Fixed effects were sex (male and female), breed (Landrace and Pietrain), and status (control and PCV2 infected). The repeated variable was time. If an overall significant difference in time was noted (the rejection level for the null hypothesis was 0.05), a 3-way factorial ANOVA was performed at each time point to determine on which specific day(s) the dependent variables had a significant effect. This procedure was done using the GLM procedure of SAS with sex, status, and breed, and all associated interactions as independent variables. Significant effects of a factor were noted as a $P < 0.05$. To summarize and simplify the histopathological data, response feature analysis was used to condense lesions scores from various tissues into a single score per animal as described (Opriessnig et al., 2004a). The GLM procedure of SAS was used on the generated lesion score as described above. To analyze the effect of the sow on the initial anti-PCV2-IgG concentrations, the GLM procedure of SAS was run separately for each breed, Pietrain and Landrace. For this analysis, the model included

effects for sow only; the anti-PCV2 concentration was considered the independent variable.

RESULTS

Clinical Evaluation and Macroscopic Lesions

None of the pigs in any of the groups developed clinical disease throughout the duration of the study. Macroscopic lesions were limited to enlarged lymph nodes and noncollapsed, mottled-tan lungs in individual pigs without statistical differences between groups.

Histopathology

In Table 1, the group means (\pm SE) for the amount of PCV2 antigen by IHC staining, degree of lymphoid depletion, and degree of histiocytic replacement in selected lymphoid tissues are summarized. In the Pietrain-PCV2 group, 65.4% had normal lymphoid tissues, 34.6% had occasional mild depletion of follicles in individual lymph nodes associated with low numbers of PCV2-antigen, and none of the pigs had moderate or severe PCV2-associated lesions. Individual Landrace pigs (30.7%), however, had moderate-to-severe lymphoid depletion and histiocytic replacement in lymphoid tissues and moderate lymphohistiocytic infiltration in liver and heart tissues. These pigs originated from 5 different sires. In comparison with Landrace-NEG and Pietrain-NEG, Landrace-PCV2 and Pietrain-PCV2 had significantly greater overall severity of lymphoid lesions scores ($P < 0.0001$; Figure 1) and more severe interstitial pneumonia scores ($P = 0.002$; data not shown). Within the PCV2-inoculated groups, Pietrain-PCV2 animals had significantly less overall severity of lymphoid lesions scores than Landrace-PCV2 animals ($P = 0.02$; Figure 1). Furthermore, 6 pigs, 5 pigs, and 1 pig in the Landrace-PCV2 group had mild, moderate, and severe lymphoid lesions, respectively. In contrast, 9 Pietrain-PCV2 animals had mild, whereas

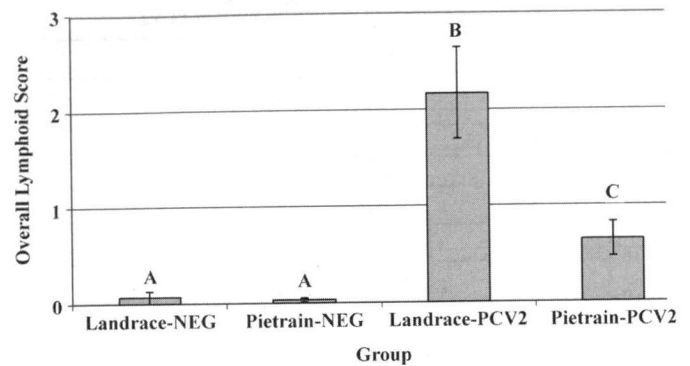


Figure 1. Mean group overall lymphoid lesion score (lymphoid depletion, histiocytic replacement, amount of porcine circovirus type 2 (PCV2)-antigen as determined by immunohistochemistry) 21 d post PCV2 inoculation. NEG = noninoculated negative control. Data are presented as group means \pm SE. ^{A-C}Columns that are connected by different letters are significantly ($P < 0.05$) different from each other.

none had moderate or severe, overall lymphoid lesions (Figure 2).

Anti-PCV2-IgM and -IgG Antibodies

All pigs had passively acquired IgG antibodies to PCV2 at arrival in the research facility at 3 wk of age. The mean-group anti-PCV2-IgG antibodies waned to negative concentrations around 12 wk of age in Pietrain pigs (at this time point 18/39 Landrace pigs and 0/39 Pietrain pigs had S/P ratios above the cut-off) and around 18 wk of age in Landrace pigs (5/39 Landrace pigs and 0/39 Pietrain pigs had S/P ratios above the cut-off). On the day of PCV2 inoculation (21 wk of age), all pigs were negative for anti-PCV2-IgM and -IgG antibodies.

Anti-porcine circovirus type 2-IgM optical densities from 0 to 21 DPI for all groups are summarized in Figure 3. The Landrace-NEG and Pietrain-NEG groups did not develop detectable anti-PCV2 antibodies throughout the study, whereas Landrace-PCV2 and Pietrain-

Table 1. Group mean (\pm SE) for porcine circovirus type 2 (PCV2) immunohistochemical staining (IHC), lymphoid depletion (LD), and histiocytic replacement (HR) in selected lymphoid tissues

Item	Landrace-NEG ¹	Pietrain-NEG	Landrace-PCV2	Pietrain-PCV2
Lymph node				
PCV2 IHC ²	0.00 (± 0.00) ^A	0.00 (± 0.00) ^A	0.54 (± 0.17) ^B	0.08 (± 0.05) ^A
LD ³	0.00 (± 0.00) ^A	0.15 (± 0.10) ^A	1.04 (± 0.21) ^B	0.23 (± 0.12) ^A
HR ³	0.00 (± 0.00) ^A	0.08 (± 0.08) ^A	0.81 (± 0.18) ^B	0.23 (± 0.10) ^A
Tonsil				
PCV2 IHC ²	0.00 (± 0.00) ^A	0.00 (± 0.00) ^A	0.42 (± 0.15) ^B	0.19 (± 0.10) ^{BA}
LD ³	0.00 (± 0.00) ^A	0.00 (± 0.00) ^A	0.46 (± 0.14) ^B	0.04 (± 0.04) ^A
HR ³	0.00 (± 0.00) ^A	0.00 (± 0.00) ^A	0.38 (± 0.14) ^B	0.04 (± 0.04) ^A
Spleen				
PCV2 IHC ²	0.00 (± 0.00) ^A	0.00 (± 0.00) ^A	0.08 (± 0.05) ^A	0.04 (± 0.04) ^A
LD ³	0.08 (± 0.08) ^A	0.00 (± 0.00) ^A	0.58 (± 0.16) ^B	0.15 (± 0.07) ^A
HR ³	0.08 (± 0.08) ^A	0.00 (± 0.00) ^A	0.50 (± 0.16) ^B	0.15 (± 0.09) ^{BA}

^{A,B}Means within row with different superscripts are significantly different ($P < 0.05$).

¹NEG = noninoculated negative control.

²Scores range from 0 (no signal) to 3 (more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining).

³Scores ranged from 0 (normal) to 3 (severe).

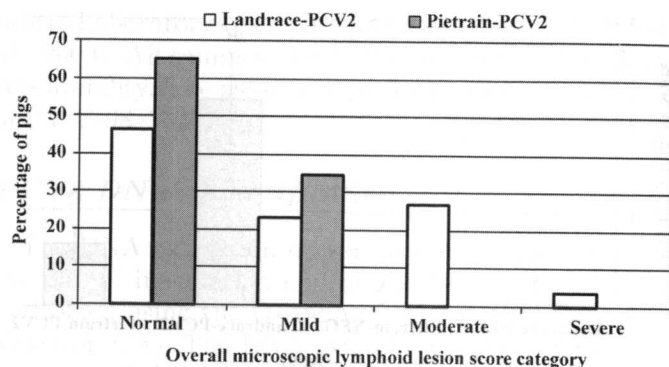


Figure 2. Percentage of Landrace and Pietrain pigs inoculated with porcine circovirus type 2 (PCV2) within the overall microscopic lymphoid lesion category normal (score = 0), mild (score = 1 to 3), moderate (score = 4 to 6), and severe (score = 7 to 9).

PCV2 groups developed anti-PCV2-IgM antibodies by DPI 14. Within the PCV2-inoculated animals, there were no significant differences between OD ratios in Landrace and Pietrain at any DPI ($P > 0.05$).

Anti-porcine circovirus type 2-IgG S/P ratios from 0 to 21 DPI for all groups are summarized in Figure 4. The Landrace-NEG and Pietrain-NEG groups did not develop anti-PCV2-IgG antibodies within the observation period, whereas Landrace-PCV2 and Pietrain-PCV2 groups seroconverted by DPI 14. Within the PCV2-inoculated animals, there were no significant differences in OD ratios between Landrace and Pietrain groups at any DPI ($P > 0.05$).

Neutralizing PCV2 Antibodies

During the observation period from the day of inoculation until 14 DPI, there were no differences in mean group anti-PCV2-neutralizing antibody concentrations between PCV2-infected groups/breeds (data not shown). On DPI 21, PCV2-infected pigs had significantly ($P < 0.001$) greater mean group concentrations of anti-PCV2 neutralizing antibodies compared with noninfected pigs regardless of breed [$\log_{10} \pm \text{SE}$: Landrace-NEG (1.06 ± 0.12); Pietrain-NEG (0.88 ± 0.06); Landrace-POS (1.68 ± 0.08); Pietrain-POS (1.78 ± 0.09)]. The correlation coefficients (r) between the amount of anti-PCV2

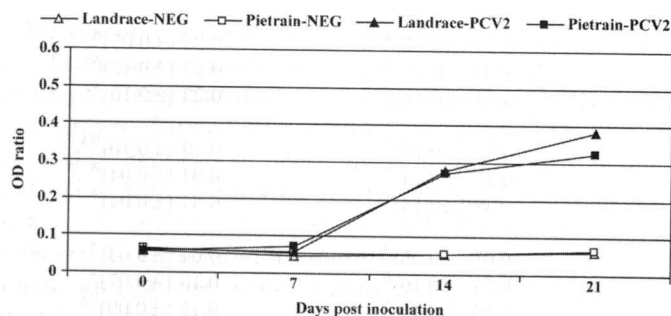


Figure 3. Mean group optical density (OD) ratios for anti-porcine circovirus type 2 (PCV2)-IgM antibody response on the day of inoculation, and at 7, 14, and 21 d postinoculation. NEG = noninoculated negative control.

neutralizing antibodies and overall severity of lymphoid lesions score were 0.12 and 0.01 for PCV2-infected pigs and noninfected pigs, respectively, regardless of breed. By breed, the Landrace-PCV2 group had a correlation coefficient of 0.31, whereas the Pietrain-PCV2 group had a correlation coefficient of 0.03.

Amount of PCV2 DNA in Serum Samples

Quantitative PCV2 PCR data for all groups is summarized in Figure 5. All pigs in all groups were PCV2 DNA negative in serum samples on the day of PCV2 inoculation at 21 wk of age. The Landrace-NEG and Pietrain-NEG groups remained PCV2 DNA negative at DPI 7, 14, and 21. Within the PCV2-inoculated animals, the amount of PCV2 DNA in serum was not significantly different between Landrace-PCV2 and Pietrain-PCV2 groups at DPI 7, 14, or 21 ($P = 0.15, 0.15$, and 0.58 , respectively). The correlation coefficients between the amount of anti-PCV2 neutralizing antibodies at DPI 21 and the amount of PCV2 DNA in serum at DPI 21 were 0.32 and 0.00 for PCV2-infected pigs and noninfected pigs, respectively, regardless of breed. The correlation coefficients between the amount of the overall severity of lymphoid lesions score and the amount of PCV2 DNA in serum at DPI 21 were 0.54 and 0.00 for PCV2-infected pigs and noninfected pigs, respectively, regardless of breed. By breed, the Landrace-PCV2 group had a correlation coefficient of 0.48, whereas the Pietrain-PCV2 group had a correlation coefficient of 0.38. The correlation coefficients between the amount of IFN- γ at DPI 21 and the amount of PCV2 DNA in serum at DPI 21 was 0.00 and 0.00 for PCV2-infected pigs and noninfected pigs, respectively, regardless of breed.

Selected Cytokine Concentrations in Plasma Samples

There were no significant differences in mean group amount of plasma IFN- γ concentrations, plasma IL-10 concentrations, and plasma IFN- α concentrations within the Landrace-PCV2 and Pietrain-PCV2 groups at DPI 21 ($P = 0.99, 0.33$, and 1.00 , respectively; data

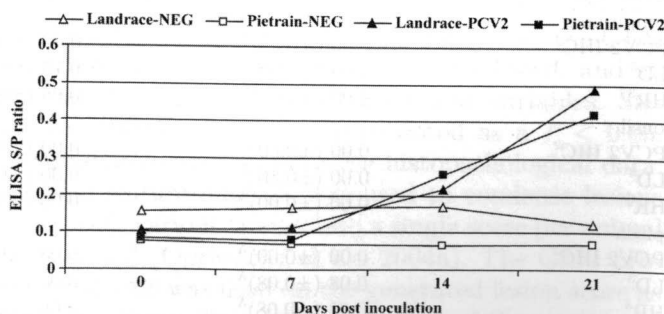


Figure 4. Mean group anti-porcine circovirus type 2 (PCV2)-IgG sample-to-positive (S/P) ratios on the day of inoculation, and at 7, 14, and 21 d postinoculation. A sample-to-positive ratio equal or above 0.2 is considered positive. NEG = noninoculated negative control.

not shown). In addition, there was no statistically significant correlation of DPI 21 plasma IFN- γ concentrations, plasma IL-10 concentrations, and plasma IFN- α concentrations and severity of lymphoid lesions in any of the pigs (data not shown).

DISCUSSION

Currently there is great interest from veterinarians, producers, and pig genetic companies to determine if a genetic host component influences PCV2 susceptibility and manifestation of PCVAD. Although effective PCV2 vaccines recently reached the market (Fachinger et al., 2008; Fort et al., 2008; Opriessnig et al., 2008), these products require additional labor and increased costs for producers. If a traceable genetic marker for PCVAD resistance were discovered, targeted breeding and genetic manipulation may facilitate reductions in PCVAD and negate the need for PCV2 vaccines in some swine herds. However, the scientific community is still at the beginning of understanding host genetic resistance to disease in pigs.

A cohort study was conducted to investigate a suspected decreased susceptibility to PCVAD in Pietrain pigs by manipulating the herd genetics via AI on 4 clinically affected farms (Rose et al., 2004). One-half of the sows were inseminated with Pietrain semen, whereas the remaining sows received the semen that was typically used on these farms. The PCV2-seroconversion, morbidity, and mortality were similar in the Pietrain offspring compared with the other pigs on these farms (Rose et al., 2004).

In Canada, 43 boars located in one boar stud were tested for the presence of PCV2 DNA in semen (McIntosh et al., 2006). Duroc and Landrace boars were found to be positive for PCV2-DNA in semen, whereas Large White and Meishan synthetic breeds did not shed PCV2 DNA in semen (McIntosh et al., 2006). On 46 swine farms in Zhejiang, China, 44.83% of all Landrace sows and 64.28% of all Landrace piglets were positive for PCV2 antibodies, values greater than observed in Yorkshire and Duroc sows (Zhou et al., 2006).

López-Soria et al. (2004) compared the effect of 3 different genetic boar lines (100% Pietrain, 50% Large White/50% Pietrain, and 25% Large White/75% Duroc) at 2 Spanish farms on the outcome of overall and PCV2-associated postweaning mortality and found a significant effect of host genetics on the manifestation of PCVAD; however, due to the limited size of the study, it could not be definitely concluded that the observed effect was due to a particular breed/line or a particular boar (López-Soria et al., 2004). Sibila et al. (2005) compared the amount of PCV2 DNA in sera from pigs in the same Spanish herds as used in the study by López-Soria et al. (2004) and found that the PCV2 DNA load correlated with genetic background and that 1 of the 3 paternal genetic lines (25% Large White/75% Duroc) had the greatest PCV2 loads in serum samples at 9 and

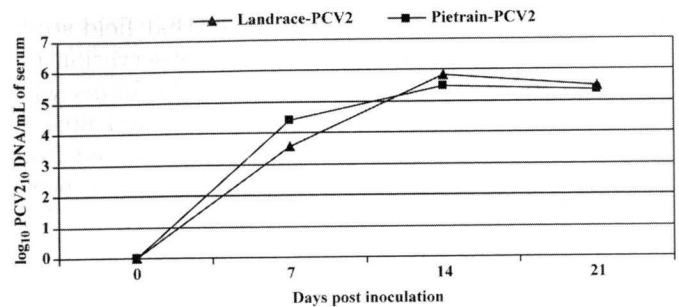


Figure 5. Amount of porcine circovirus type 2 (PCV2) genomic copies per milliliter of serum in PCV2-inoculated groups on the day of inoculation, and at 7, 14, and 21 d postinoculation. Data are presented as log₁₀ transformed group means.

21 wk of age (Sibila et al., 2005). Overall, it needs to be considered that a genetic component most likely is not confined to a certain breed or gene locus and that not all lines within a breed express all necessary genes.

By expanding our previous study (Opriessnig et al., 2006a), we tested additional pig breeds and further confirmed that differences in susceptibility to PCV2-associated lesions exist between pigs of different genetic background. This controlled study further confirms a genetic component in susceptibility to PCV2 infection and disease. In the present study, experimental PCV2 inoculation was done at 21 wk of age. This is relatively late in the production cycle and past the time at which pigs are most likely naturally exposed to PCV2 in the field; however, Landrace pigs still developed moderate-to-severe PCV2-associated lesions. One of the Landrace pigs had lesions compatible with PMWS (overall lymphoid lesion score of 7), and 2 Landrace pigs had moderate PCV2-associated lesions (overall lymphoid lesion score of 6). No clinical signs were observed in the present study; however, this is not surprising because clinical signs in pigs infected by PCV2 alone are rarely produced. The observed lesions were similar to lesions seen in cases of naturally occurring or experimentally induced systemic PCVAD in much younger animals. This implies that the assumption that older pigs are more resistant to PCVAD may be wrong and that expression of disease and lesions is more related to timing of infection.

Interestingly, although the pigs originated on the same farm, maternal anti-PCV2-antibodies waned in the Pietrain pigs by approximately 12 wk of age, whereas the majority of the Landrace pigs remained PCV2 seropositive until 18 wk of age and beyond. It has been estimated previously that the mean antibody half-life of passively derived PCV2 antibodies is approximately 19 d (Opriessnig et al., 2004b), which implies that the Landrace piglets received considerably greater concentrations of anti-PCV2-antibodies via the colostrum compared with the Pietrain piglets. Alternatively, the anti-PCV2-antibodies in Landrace pigs may have been degraded more slowly than in the Pietrain pigs.

It has been demonstrated that maternally derived anti-PCV2-antibodies are protective from developing

clinical PCVAD (Allan et al., 2002). In that field study and under study conditions during the observation period, none of the piglets derived from the dams with high PCV2-antibodies developed PMWS, and 60% of the piglets that did develop PMWS were derived from the dams with the least PCV2 serum antibody concentrations (Allan et al., 2002). Another European field study investigated the effect of the sow on PMWS in a cohort study in 3 sow herds with high postweaning mortality due to PMWS (Hassing et al., 2003). A total of 1,226 piglets derived from 122 sows were included in this study. It was found that the offspring from sows with increased antibody concentrations against PCV2 at farrowing had a greater risk of dying after weaning. The risk of dying was also dependent on increasing titers against PCV2 from weaning until 4 wk after weaning (Hassing et al., 2003). Furthermore, it has been previously shown that decreased concentrations of passively acquired antibodies do not prevent PCV2 replication (McKeown et al., 2005) and subclinically infected pigs may serve as a continuous source of PCV2.

It can be speculated that pigs that are more susceptible to PCVAD, but survive the growing phase, have greater amounts of antibodies and therefore pass on greater concentrations of colostral-antibodies to their offspring. Interestingly, the data in our study are supported by a recent Chinese field study that found a trend toward greater prevalence of anti-PCV2-antibodies in Landrace sows compared with Yorkshire and Duroc sows (Zhou et al., 2006).

The mechanism of progression of PCV2 infection to clinical disease and the pathogenesis of PCV2-infection in general remain poorly understood. We previously investigated the immune gene expression profiles in tracheobronchial lymph nodes after experimental infection with PCV2, *Mycoplasma hyopneumoniae*, and PCV2/*M. hyopneumoniae* coinfection and compared the profiles with those of uninfected controls (Opriessnig et al., 2006b). We found that single PCV2 infection was characterized by significantly increased IFN- γ and IL-8 chemokine gene expression and speculated that upregulation of certain cytokines during the course of inflammation above a certain level would influence the concentration and type of antibodies produced in response to infection, resulting in PCVAD (Opriessnig et al., 2006b). Inappropriate and ineffective cytokine production could be associated with failure to produce sufficient concentrations of neutralizing antibodies to control the PCV2. One possible mechanism of this failure is by a lack of isotype switching of IgM antibodies to IgG antibodies. The IFN- γ stimulates expression of both MHC class I and II molecules and co-stimulates and promotes B cell switching to certain Ig subclasses, and concurrently inhibits the antibodies associated with a T helper 2-like antibody response, which would include less efficacious antibodies such as IgE. In our current study, we did not find a breed-dependent difference in expression of selected cytokines, nor did we find

a correlation with plasma concentrations of selected cytokines and overall lymphoid lesions scores. Plasma IFN- γ , IL-10, and IFN- α concentrations were not different in pigs with moderate-to-severe overall lymphoid lesions compared with control pigs. The lack of overall plasma cytokine correlation is in contrast to previous field observations (Darwich et al., 2003; Sipos et al., 2004); a likely explanation would be the lack of concurrent pathogens in our controlled experimental study. In addition, these pigs were infected at 21 wk of age when their immune system was more fully developed.

It has recently been demonstrated that PCVAD-affected pigs have decreased concentrations of neutralizing antibodies compared with clinically unaffected pigs (Meerts et al., 2006). Neutralizing antibodies were detected between 15 d (Meerts et al., 2005, 2006) and 28 d (Pogranichnyy et al., 2000) post PCV2 infection and were correlated with protection or clearance of PCV2 infection in gnotobiotic pigs (Meerts et al., 2006). One possible mechanism of the lack of serum neutralizing antibody production is through impaired antibody isotype switching. The PCV2-specific IgM antibodies have been shown to appear within the first few weeks after PCV2 infection, followed by isotype switching to predominantly IgG production starting at approximately 14 d post PCV2 infection. Pigs that develop PCVAD have been shown to remain seronegative for PCV2-specific IgG (Opriessnig et al., 2004a), suggesting that the normal switching process has been circumvented. In this study, we tested neutralizing antibodies to determine if differences in neutralizing antibody concentrations could be correlated with breed and differences in disease susceptibility. Differences in anti-PCV2 serum neutralizing antibody concentrations were not observed between the 2 breeds. Furthermore, there was no apparent correlation with overall lymphoid lesion severity and amount of anti-PCV2 serum neutralizing antibodies. This may be due to the timing of necropsy (21 DPI), which may have been too early in the course of neutralizing antibody induction and production for demonstrating significant differences between groups. Interestingly, the pigs with the greatest amount of anti-PCV2 neutralizing antibodies at the time of necropsy had an overall lymphoid score of zero, whereas the pig with the greatest overall lymphoid score (score = 7) was negative for anti-neutralizing antibodies.

In summary, the results from this study indicated that a sampled group of Landrace pigs are more susceptible to PCV2-associated lesions. We also demonstrated that a sampled group of Pietrain pigs are less susceptible to PCV2-associated lesions, which supports anecdotal reports from the field. The body of evidence to support the need for more detailed genetic work in this area continues to grow. Use of new genomic technologies should be used to determine if individual alleles or groups of alleles from several genes differ between breeds and are associated with the apparent breed differences seen here and in other studies.

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